recites "[l]inker arms may include alkylene groups of 1 to 12 carbon atoms, alkenylene groups of 2 to 12 carbon atoms and 1 or 2 olefinic bonds, alkynylene groups of 2 to 12 carbon atoms and 1 or 2 acetylenic bonds, or such groups substituted at a terminal point with nucleophilic groups such as oxy, thio, amino or chemically blocked derivatives thereof..."

Claims 21 and 22 are directed to compounds having a pyrimidine base moiety and modified as noted above for the pyrazolo[3,4-d]pyrimidines (see, for example, Schemes 1 and 2, columns 7 and 8). The specification at column 7, lines 22-40, notes that the invention is directed to two classes of compounds that have demonstrated particular usefulness upon incorporation into oligonucleotides. The first class of compounds are the pyrimidine derivatives, the preparation of which is shown in Schemes 1 and 2. A comparison of the side chain components finds that these schemes illustrate the incorporation of alkynylene, alkenylene and alkylene chains which terminate in Y', a group useful for the attachment of A'. Accordingly, Applicants have added claims 21 and 22 directed to these intermediates, but reducing the scope in view of, for example, Robins et al., Can. J. Chem. 60:554 (1982) and J. Org. Chem. 48:1854 (1983), and Ward et al., U.S. Pat. No. 4,711,955 references of record in the parent application.

Claims 23-28 are directed to oligonucleotides incorporating the modified bases provided in claims 16-22. Support for these claims can be found in those areas provided above for the nucleotide units and in the statement beginning at column 7, line 22, that "[t]wo classes of modified 2'deoxynucleosides have demonstrated particular usefulness in the present invention *for incorporation into oligonucleotides* ..." (emphasis added).

New claims 29-30 and 31-32 are dependent on claims 10 and 13, respectively and recite the limitations wherein the reporter group is selected from ^{3}H, ^{125}I, ^{35}S, ^{14}C and ^{32}P (see column 14, lines 1-4), and more preferably, ^{3}H.

New claims 33-44 are directed to the compounds and oligonucleotides having bases and labels as provided above, but having linking groups including the unsaturated linking groups (e.g., alkenylene and alkynylene). More particularly, claim 33 recites a labeled oligonucleotide having at least one pyrazolo[3,4-d]pyrimidine nucleotide unit with a reporter group (A) attached via a linker (W). Support for this group of embodiments can be found at column 9, lines 28-60, wherein W is a chemical linker arm (see column 9, line 51, and column

10, lines 33-35) and A is a reporter group (see column 9, lines 53-55). The portion designated in the specification as $-(X)_n-$ is not depicted in the claimed formula as the subscript n is 0 in this group of embodiments. Claims 34 and 35 recites certain radiolabels (see column 14, lines 1-4).

In a related group of embodiments, claims 36-38 recite similar labeled oligonucleotides in which the label is pendent to a pyrimidine base, e.g., as depicted in column 7. In order to be consistent with claims 33-35, the pendent linker and label have been depicted as -W-A, in which W and A have the same definitions as provided above. Applicants believe the equivalent scope sought for linking group and reporter group in this series of claims would be apparent to one of skill in the art upon viewing the general structure, Schemes 1 and 2, and the recitation that two classes of modified bases have demonstrated usefulness in the present invention.

Claims 39-41 and 42-44 recite groups of compounds (pyrazolo[3,4-d]pyrimidines and pyrimidines, respectively) having attached reporter groups. More particularly, these claims are directed to the monomers used in the oligonucleotides provided in claims 33-38. Support in the specification can be found as outlined above.

Applicants believe no new matter is presented in any portion of the requested amendments.

Concerning the sequence listings contained in the specification, the Office's attention is respectfully directed to the enclosed Cross-Reference under 37 C.F.R. § 1.821(e); and Statement under 37 C.F.R. §§ 1.821 (f) and (g).

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

WHAT IS CLAIMED IS:

1. (Amended) An oligonucleotide having at least one nucleotide of the formula

$$R_1\text{-}B\text{-}(CH_2)_q\text{-}(Y)_r\text{-}(CH_2)_m\text{-}A'$$

wherein

R_1 is a 1-(β-D-ribofuranosyl) or 1-(β-D-2-deoxyribofuranosyl) group which is optionally substituted on one or more of its hydroxyl functions with a Z group, wherein Z independently is methyl or a phosphate, thiophosphate, alkylphosphate or alkanephosphonate group;

B is a heterocyclic base selected from purine and pyrazolo[3,4-d]pyrimidine groups wherein the $(CH_2)_q$ group is attached to the 7-position or 8 position of the purine and 3-position of the pyrazolo[3,4-d]pyrimidine groups and the R_1 group is attached to the 9-position of the purine and to the 1-position of the pyrazolo[3,4-d]pyrinidine groups;

Y is a functional linking group selected from a group consisting of –O-, -S-, -NR'-, -NH-CO-, trifluoroacetamido and [phtalimido] phthalimido groups where R' is H or $C_{1\text{-}6}$ alkyl, and at least one of the $(CH_2)_m$ and $(CH_2)_q$ groups is directly linked to the –O-, -S-, -NR'-, NH-CO-, trifluoroacetamido and [phtalimido] phthalimido groups and the other of said $(CH_2)_m$ and $(CH_2)_q$ groups is linked to the heterocyclic base with a carbon to carbon bond;

m is 1 to 8, inclusive;

q is 0 to 8, inclusive;

r is 0 or 1; and

A' is a group selected from chloro, bromo, iodo, SO_2R''', $S^+R'''R''''$ and a radical which activates the carbon to which it is attached for nucleophilic substitution, where each of R''' and R'''' is independently $C_{1\text{-}6}$ alkyl or aryl or R''' and R'''' together form a $C_{1\text{-}6}$ alkylene bridge.

2. An oligonucleotide according to claim 1 wherein B is selected from adenine-8-yl, guanine-8-yl, 4-aminopyrazolo[3,4-d]pyrimidin-3-yl, and 4-amino-6-oxopyrazolo[3,4-d]pyrimidin-3-yl groups.

3. An oligonucleotide according to claim 1 wherein m is 1, 2 or 3; q is 2, 3, or 4; and r is 1.

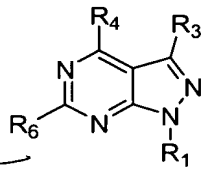
4. An oligonucleotide according to claim 1 wherein the R_1 group is 1-(β-D-ribofuranosyl).

5. An oligonucleotide according to claim 1 wherein the R_1 group is 1-(β-D-2-deoxyribofuranosyl).

6. An oligonucleotide according to claim 1 wherein the R_1 group is 1-(β-D-2-O-methyl-ribofuranosyl).

7. An oligonucleotide according to claim 1 wherein the group $-(CH_2)_q-(Y)_r-(CH_2)_m-A'$ is 3-iodoacetamidopropyl, 3-(4-bromobutyramido)propyl, 4-iodoacetamidobutyl, or 4-(4-bromobutyramido)butyl.

8. (Amended) A compound of the formula



where R_1 is H, or a 1-(β-D-ribofuranosyl) or 1-(β-D-2-deoxyribofuranosyl) group which is optionally substituted on one or more of its hydroxyl functions with a Z group wherein Z independently is methyl or a phosphate, thiophosphate, alkylphosphate or alkanephosphonate group, or a reactive precursor of said phosphate, thiophosphate, alkylphosphate or alkanephosphonate group which precursor is suitable for internucleotide bond formation;

R_3 is $(CH_2)_q-(Y)_r-(CH_2)_m-A''$ where A'' is a group selected from chloro, bromo, iodo, SO_2R''', $S^+R'''R''''$ and a radical which activates the carbon to which it is attached for nucleophilic substitution, where each of R''' and R'''' is independently C_{1-6} alkyl or aryl or R''' and R'''' together form a C_{1-6} alkylene bridge, or A'' is an intercalator group, a metal ion chelator or a reporter group;

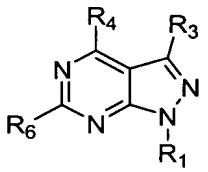
Y is a functional linking group selected from a group consisting of –O-, -S-, -NR'-, -NH-CO-, trifluoroacetamido and [phthalimido] phthalimido groups where R' is H or C_{1-6} alkyl, and at least one of the $(CH_2)_m$ and $(CH_2)_q$ groups is directly linked to said –O-, -S-, -NR'-, NH-CO-, trifluoroacetamido and [phthalimido] phthalimido groups and the other of said $(CH_2)_m$ and $(CH_2)_q$ groups is linked to the heterocyclic base with a carbon to carbon bond;

each of m and q is independently 0 to 8, inclusive; r is 0 or 1 provided that when A" is a group selected from chloro, bromo, iodo, SO_2R''', $S^+R'''R''''$ and a radical which activates the carbon to which it is attached for nucleophilic substitution, then m is not 0;

each of R_4 and R_6 is independently H, OR, SR, NHOR, NH_2, or $NH(CH_2)_tNH_2$ where R is H or C_{1-6}alkyl and t is an integer from 0 to 12.

9. A compound in accordance with claim **8** where each of R_4 and R_6 is independently selected from a group consisting of H, OH and NH_2.

10. A compound of the formula



where R_1 is H, or a 1-(β-D-ribofuranosyl) or 1-(β-D-2-deoxyribofuranosyl) group which is optionally substituted on one or more of its hydroxyl functions with a Z group wherein Z independently is methyl or a phosphate, thiophosphate, alkylphosphate or alkanephosphonate group, or a reactive precursor of said phosphate, thiophosphate, alkylphosphate or alkanephosphonate group which precursor is suitable for internucleotide bond formation;

R_3 is $(CH_2)_q$-$(Y)_r$-$(CH_2)_m$-A" where A" is a reporter group;

Y is a functional linking group selected from a group consisting of –O-, -S-, -NR'-, -NH-CO-, trifluoroacetamido and [phtalimido] phthalimido groups where R' is H or C_{1-6} alkyl, and at least one of the $(CH_2)_m$ and $(CH_2)_q$ groups is directly linked to said –O-, -S-, -NR'-, NH-CO-, trifluoroacetamido and [phtalimido] phthalimido groups and the other of said $(CH_2)_m$ and $(CH_2)_q$ groups is linked to the heterocyclic base with a carbon to carbon bond;

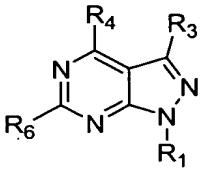
each of m and q is independently 0 to 8, inclusive; r is 0 or 1, and

each of R_4 and R_6 is independently H, OR, SR, NHOR, NH_2, or $NH(CH_2)_tNH_2$ where R is H or C_{1-6}alkyl and t is an integer from 0 to 12.

11. A compound in accordance with claim **10** where each of R_4 and R_6 is independently selected from a group consisting of H, OH and NH_2.

12. A compound in accordance with claim **11** where the reporter group is biotin or 2,4-dinitrobenzene.

13. An oligonucleotide having at least one nucleotide of the formula



where R_1 is a 1-(β-D-ribofuranosyl) or 1-(β-D-2-deoxyribofuranosyl) group which is optionally substituted on one or more of its hydroxyl functions with a Z group wherein Z independently is methyl or a phosphate, thiophosphate, alkylphosphate or alkanephosphonate group;

R_3 is $(CH_2)_q$-$(Y)_r$-$(CH_2)_m$-A and A is a reporter group;

Y is a functional linking group selected from a group consisting of –O-, -S-, -NR'-, -NH-CO-, trifluoroacetamido and [phtalimido] phthalimido groups where R' is H or C_{1-6} alkyl, and at least one of the $(CH_2)_m$ and $(CH_2)_q$ groups is directly linked to said –O-, -S-, -NR'-, NH-CO-, trifluoroacetamido and [phtalimido] phthalimido groups and the other of said $(CH_2)_m$ and $(CH_2)_q$ groups is linked to the heterocyclic base with a carbon to carbon bond;

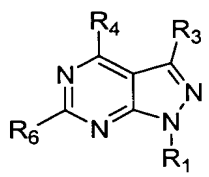
each of m and q is independently 0 to 8, inclusive; r is 0 or 1, and

each of R_4 and R_6 is independently H, OR, SR, NHOR, NH_2, or $NH(CH_2)_tNH_2$ where R is H or C_{1-6}alkyl and t is an integer from 0 to 12.

14. An oligonucleotide in accordance with claim **13** where each of R_4 and R_6 is independently selected from a group consisting of H, OH and NH_2.

15. An oligonucleotide in accordance with claim **14** where the reporter group is biotin or 2,4-dinitrobenzene.

16. (New) A compound having the formula



wherein R_1 is H, or a 1-(β-D-ribofuranosyl) or 1-(β-D-2-deoxyribofuranosyl) group which is optionally substituted on one or more of its hydroxyl functions with a Z group wherein Z independently is methyl or a phosphate, thiophosphate, alkylphosphate or alkanephosphonate group which precursor is suitable for internucleotide bond formation;

R_3 is –W-X, wherein W is a chemical linker arm selected from the group consisting of C_{1-12} alkylene, C_{2-12} alkenylene and C_{2-12} alkynylene, and X is selected from the group consisting of OH, SH, NH_2 and chemically blocked derivatives thereof;

each of R_4 and R_6 is independently H, OR, SR, NHOR, NH_2, or $NH(CH_2)_tNH_2$ where R is H or C_{1-6}alkyl and t is an integer from 0 to 12

with the proviso that when W is $–CH_2CH_2$-, then X is other than NH_2.

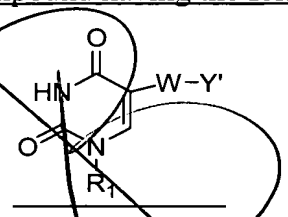
17. (New) A compound of claim **16**, wherein W is C_{1-12} alkylene and X is selected from the group consisting of OH, NH_2 and chemically blocked derivatives thereof.

18. (New) A compound of claim **16**, wherein W is C_{2-12} alkynylene and X is selected from the group consisting of OH, NH_2 and chemically blocked derivatives thereof.

19. (New) A compound of claim **17**, wherein W is pentyl and X is NH-trityl.

20. (New) A compound of claim **16**, wherein R_4 is NH_2 or OH and R_6 is H or NH_2

21. (New) A compound having the formula

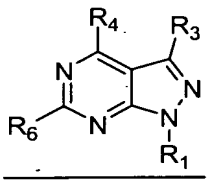


wherein R_1 is a 1-(β-D-ribofuranosyl) or 1-(β-D-2-deoxyribofuranosyl) group which is optionally substituted on one or more of its hydroxyl functions with a Z group wherein Z independently is methyl or a phosphate, thiophosphate, alkylphosphate or alkanephosphonate group which precursor is suitable for internucleotide bond formation;

W is a chemical linker arm selected from the group consisting of C_{2-12} alkynylene, and Y' is selected from the group consisting of OH, SH and chemically blocked derivatives thereof.

22. (New) A compound of claim 21, wherein Y' is selected from the group consisting of OH and chemically blocked derivatives thereof.

23. (New) An oligonucleotide comprising at least one nucleotide unit of the formula



wherein R_1 is a 1-(β-D-ribofuranosyl) or 1-(β-D-2-deoxyribofuranosyl) group which is optionally substituted on one or more of its hydroxyl functions with a Z group wherein Z independently is methyl or a phosphate, thiophosphate, alkylphosphate or alkanephosphonate group which precursor is suitable for internucleotide bond formation;

R_3 is –W-X, wherein W is a chemical linker arm selected from the group consisting of C_{1-12} alkylene, C_{2-12} alkenylene and C_{2-12} alkynylene, and X is selected from the group consisting of OH, SH, NH_2 and chemically blocked derivatives thereof;

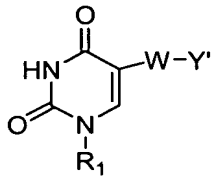
each of R_4 and R_6 is independently H, OR, SR, NHOR, NH_2, or $NH(CH_2)_tNH_2$ where R is H or C_{1-6}alkyl and t is an integer from 0 to 12.

24. (New) An oligonucleotide of claim 23, wherein W is C_{1-12} alkylene and X is selected from the group consisting of OH, NH_2 and chemically blocked derivatives thereof.

25. (New) An oligonucleotide of claim 23, wherein W is C_{2-12} alkynylene and X is selected from the group consisting of OH, NH_2 and chemically blocked derivatives thereof.

26. (New) An oligonucleotide of claim 24, wherein W is pentyl and X is NH-trityl.

27. (New) An oligonucleotide comprising at least one nucleotide unit of the formula



wherein R_1 is a 1-(β-D-ribofuranosyl) or 1-(β-D-2-deoxyribofuranosyl) group which is optionally substituted on one or more of its hydroxyl functions with a Z group wherein Z independently is methyl or a phosphate, thiophosphate, alkylphosphate or alkanephosphonate group which precursor is suitable for internucleotide bond formation;

W is a chemical linker arm selected from the group consisting of C_{2-12} alkynylene, and Y' is selected from the group consisting of OH, SH and chemically blocked derivatives thereof.

28. (New) An oligonucleotide of claim 27, wherein Y' is selected from the group consisting of OH and chemically blocked derivatives thereof.

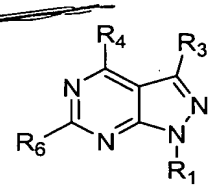
29. (New) A compound of claim **10**, wherein the reporter group is selected from the group consisting of ^{3}H, ^{125}I, ^{35}S, ^{14}C and ^{32}P.

30. (New) A compound of claim **10**, wherein the reporter group is ^{3}H.

31. (New) An oligonucleotide of claim **13**, wherein the reporter group is selected from the group consisting of ^{3}H, ^{125}I, ^{35}S, ^{14}C and ^{32}P.

32. (New) An oligonucleotide of claim **13**, wherein the reporter group is ^{3}H.

33. (New) A labeled oligonucleotide comprising at least one nucleotide unit of the formula



wherein R_1 is a 1-(β-D-ribofuranosyl) or 1-(β-D-2-deoxyribofuranosyl) group which is optionally substituted on one or more of its hydroxyl functions with a Z group wherein Z independently is methyl or a phosphate, thiophosphate, alkylphosphate or alkanephosphonate group which precursor is suitable for internucleotide bond formation;

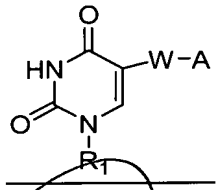
R_3 is –W-A, wherein W is a chemical linker arm selected from the group consisting of C_{1-12} alkylene, C_{2-12} alkenylene and C_{2-12} alkynylene, and A is a reporter group; and

each of R_4 and R_6 is independently H, OR, SR, NHOR, NH_2, or $NH(CH_2)_tNH_2$ where R is H or C_{1-6}alkyl and t is an integer from 0 to 12.

34. (New) A labeled oligonucleotide of claim 33, wherein said reporter group is selected from the group consisting of ^{3}H, ^{125}I, ^{35}S, ^{14}C and ^{32}P.

35. (New) A labeled oligonucleotide of claim 33, wherein said reporter group is ^{3}H.

36. (New) A labeled oligonucleotide comprising at least one nucleotide unit of the formula



wherein R_1 is a 1-(β-D-ribofuranosyl) or 1-(β-D-2-deoxyribofuranosyl) group which is optionally substituted on one or more of its hydroxyl functions with a Z group wherein Z independently is methyl or a phosphate, thiophosphate, alkylphosphate or alkanephosphonate group which precursor is suitable for internucleotide bond formation;

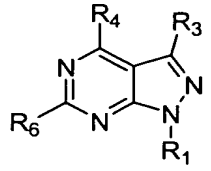
W is a chemical linker arm selected from the group consisting of C_{2-12} alkynylene; and

A is a reporter group.

37. (New) A labeled oligonucleotide of claim 36, wherein said reporter group is selected from the group consisting of ^{3}H, ^{125}I, ^{35}S, ^{14}C and ^{32}P.

38. (New) A labeled oligonucleotide of claim 36, wherein said reporter group is ^{3}H.

39. (New) A compound having the formula



wherein R_1 is H, or a 1-(β-D-ribofuranosyl) or 1-(β-D-2-deoxyribofuranosyl) group which is optionally substituted on one or more of its hydroxyl functions with a Z group wherein Z independently is methyl or a phosphate, thiophosphate, alkylphosphate or alkanephosphonate group which precursor is suitable for internucleotide bond formation;

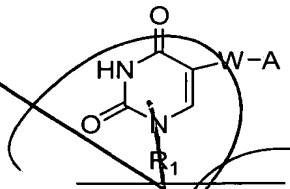
R_3 is –W-A, wherein W is a chemical linker arm selected from the group consisting of C_{1-12} alkylene, C_{2-12} alkenylene and C_{2-12} alkynylene, and A is a reporter group; and

each of R_4 and R_6 is independently H, OR, SR, NHOR, NH_2, or $NH(CH_2)_tNH_2$ where R is H or C_{1-6}alkyl and t is an integer from 0 to 12.

40. (New) A compound of claim 39, wherein said reporter group is selected from the group consisting of ^{3}H, ^{125}I, ^{35}S, ^{14}C and ^{32}P.

41. (New) A compound of claim 39, wherein said reporter group is ^{3}H.

42. (New) A compound having the formula



wherein R_1 is a 1-(β-D-ribofuranosyl) or 1-(β-D-2-deoxyribofuranosyl) group which is optionally substituted on one or more of its hydroxyl functions with a Z group wherein Z independently is methyl or a phosphate, thiophosphate, alkylphosphate or alkanephosphonate group which precursor is suitable for internucleotide bond formation;

W is a chemical linker arm selected from the group consisting of C_{2-12} alkynylene; and

A is a reporter group.

43. (New) A compound of claim **42**, wherein said reporter group is selected from the group consisting of ^{3}H, ^{125}I, ^{35}S, ^{14}C and ^{32}P.

44. (New) A compound of claim **42**, wherein said reporter group is ^{3}H.

Q3 concl'd.